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Separation of two sympatric sibling species of *Formica* L. ants by a behavioural choice test based on brood discrimination

Abstract. Recent work by PAMILO et al. (1992) has shown that nests of *Formica lugubris* ZETT. occurring within the same area of the Jura mountains can be divided into two genetically distinct types, most likely representing two sibling species. The issue of the present work is to check whether those genetical types or taxa can be separated by a behavioural choice test based on discrimination of sexual brood. Workers of artificial nests were offered a choice between non-nestmate pupae of their own type and pupae of the alien type. They showed in most tests a significant preference for non-nestmate pupae of their own electrophoretic type. The results are discussed both with reference to the nature of the discrimination cues involved and to the speciation process in wood ants.

INTRODUCTION

BREED and BENNETT (1987), in their survey of kin recognition in highly eusocial insects, pointed out two research themes as emerging: "1. Testing whether the cues used in recognition have an environmental or endogenous (to the colony) source. 2. Determining if there is a correlation between the phenotype and the genotype for those cues that are endogenous". The research themes outlined by BREED and BENNETT have proved stimulating but also difficult.

One of the main results, so far, is that data sets based on biochemical phenotypic characters and genetic markers have been found to be highly concordant in fire ants (ROSS et al. 1987). These gas-chromatographic patterns of fire ants represent the same type of volatile hydrocarbons generally used for olfactory communication in ants. The results of ROSS et al. (1987) lead therefore to some optimism concerning the possibility of getting an answer to the behavioural questions raised by BREED and BENNETT.

Our aim was to find out whether semiochemical phenotypes in wood ants, as measured by the behavioural method, the "pupae carrying test" (ROSENGREN, CHERIX 1981), are concordant with allozyme markers studied by electrophoresis (e.g. PAMILO et al. 1978). We intended to study a single species for that purpose but it soon appeared that our observations of two electrophoretic types of *Formica lugubris* ZETT. most likely represented two sympatric sibling species (PAMILO et al. 1992).

The basic research themes formulated by BREED and BENNETT are also relevant to species recognition. But we also have to take into consideration the possible mechanisms of speciation in polygynous populations of the *Formica rufa* group – one of the interests of the late Professor Bohdan PISARSKI.

The frequent occurrence of polytypy and sibling species in ants is thought to be primarily an allopatric process (WARD 1989). WARD mentions a few possible exceptions to that rule, including problematic cases within the *F. rufa* group mentioned by VEPSÄLÄINEN and PISARSKI (1981).

The recent finding of two *F. rufa* group sibling species occurring within the same area of the Jura mountains in Switzerland demonstrates, if not sympatric speciation, at least the maintenance of closely related taxa under sympatric conditions (PAMILO et al. 1992).

How can those two species, or alleged species, maintain genetical isolation in the absence of geographical barriers? Differences in chemical signals used for mating (WALTER et al. 1993) are the first clue which comes to mind. However, speciation in ants is probably not only a sexual but also a social affair. Social recognition and discrimination cues, e.g. those used by workers when distinguishing between their "own" and "alien" brood, may also be important, especially in the polycalic taxa discussed here. Polycaly implies that the ants exchange brood along communication routes connecting different nests of a supercolony (CHERIX 1980) and is therefore one aspect of the gene flow within a population.

However, the first question to be asked is not whether this or that mechanism may have contributed to the biological barrier, but to ascertain that any differences in genetically mediated recognition cues between the alleged sibling species exist, and can be demonstrated behaviourally in any context. We here ask the ants themselves whether they can distinguish between one another or not.

MATERIAL AND METHODS

Study area and ants

Our study area in the Swiss Jura mountains, and the supercolonies of *F. lugubris* occurring in those highland habitats, have been described by GRIS and CHERIX (1977), CHERIX (1980, 1987) and CHERIX and BOURNE (1980). The location and code of the nests used for the present experiments are given in Fig. 1.

The ants' nests were divided into two types using the enzyme-electrophoretic study by PAMILO et al. (1992). We here use their symbols, A and B, to denote the two genetic types. The types have so far proved to be morphologically indistinguishable and both can be classified as *F. lugubris* according to the criteria of classical taxonomy. Nests of both types occur in the same area (Fig. 1, see also Fig. 4 in PAMILO et al. 1992), but do not share the same gene pool and thus they most likely represent two biological species (PAMILO et al. 1992).

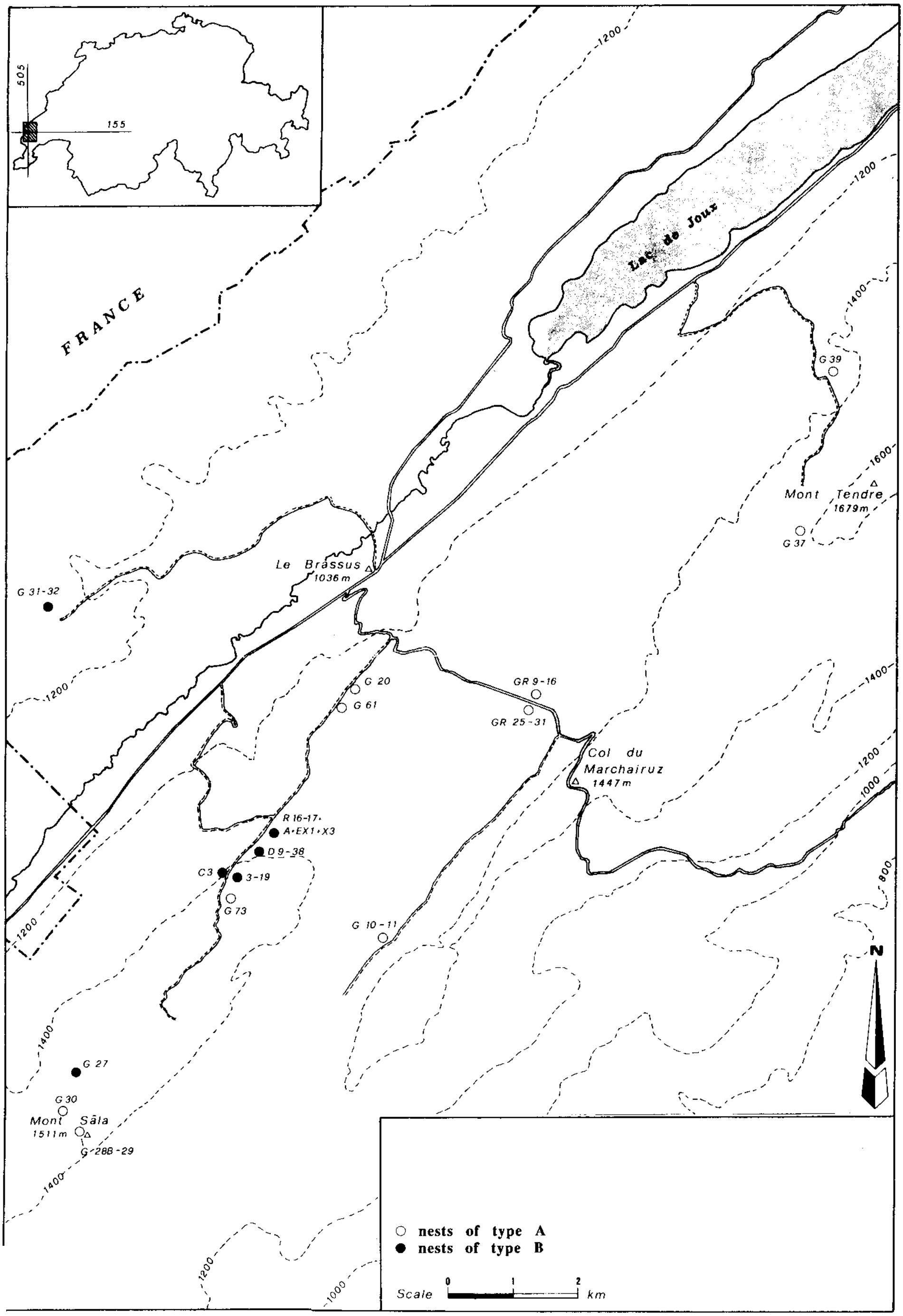


Fig. 1. A map of the study area in the Jura mountains with the location of the nests of the two electrophoretic types A and B used in the present study. Each point refers either to a single nest or to a group of connected nests. An inserted map of Switzerland shows the location of the study area.

Ants from most individual nests used as recipients and/or donors had been genetically analysed but we also included some nests which belonged to the same polycalic colony as the nests analysed. The six enzyme loci studied allowed, as a rule, a clearcut classification of our nests as either belonging to the type A or the type B, but there were some exceptions to this. Tests which included genetically ambiguous donors or recipients were later excluded from consideration.

Methods

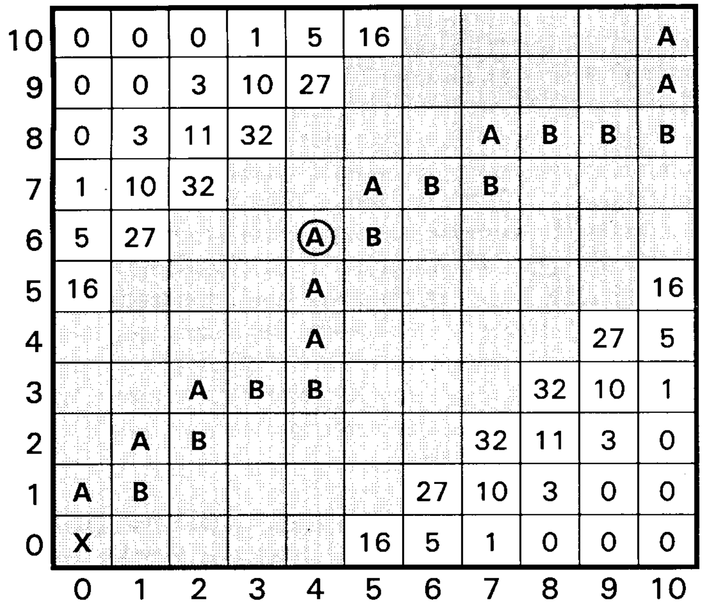
Behavioural measures of recognition/discrimination cues in ants are often based on the level of aggression between workers. This is certainly a good method if the aim is to study nestmate recognition or the ability of the ants to discriminate between conspecifics from different supercolonies. However, aggression tests, although recommended as a "taxonomic tool" in ants of the *F. rufa* group (LE MOLI, MORI 1986) proved less suitable in the present study. The variance of aggression scores in encounters between workers representing the same electrophoretic type of *F. lugubris* proved to be extremely high, ranging from fighting and killing to complete acceptance of non-nestmate workers (unpubl.). The variable outcomes of discrimination against conspecific non-nestmates is thus likely to swamp any consistent effects attributable to taxonomic or genetical differences.

Instead of aggression we used brood discrimination, as measured by the "pupae-carrying-test" developed by ROSENGREN and CHERIX (1981). With this method, *Formica* workers do not discriminate among conspecific pupae from the same local population, but may do so against pupae from remote geographical areas (ROSENGREN, CHERIX 1981, SEEGER 1985).

Our method is based on the natural reaction of carrier ants of the nurse category when faced with a choice between their "own" and "alien" pupae. The pupae are placed on a lighted arena, 22 cm in diameter, and are carried away by the ants from the arena into an artificial nest, placed under the arena and filled with natural nest material. The ants inside the dark nest enter the arena through a hole in its center (for further descriptions, see ROSENGREN, CHERIX 1981).

The arena was divided into 20 numbered sectors (10 even and 10 odd numbers). We place pupae of one kind in the odd-numbered sectors and of another in the even-numbered ones (see Fig. 1 in ROSENGREN, CHERIX 1981). The sequence in which the ants carry pupae from the sectors is noted, and the probability of a given sequence is calculated from a matrix based on a hypergeometric distribution. A probability matrix based on hypergeometric equations was designed by ROSENGREN and CHERIX (1981) but we here (Fig. 2) use a slightly different version based on SEEGER (1985). Only sequences reaching the unshaded area of the upper left or lower right corner of the matrix (Fig. 2) were considered statistically significant. Thus, a statistically significant result may be achieved even if the ants do not remove all pupae from the arena.

The data analysis produced two results. First, we assessed the level of statistical significance of individual experiments according to the probabilities given in the matrix described above. The expression "preference" is used in the text when the significance level $p \leq 0.032$ (note that this is the highest probability value shown in



10	0	0	0	1	5	16					A
9	0	0	3	10	27						A
8	0	3	11	32				A	B	B	B
7	1	10	32			A	B	B			
6	5	27			Ⓐ	B					
5	16				A						16
4					A					27	5
3			A	B	B				32	10	1
2		A	B					32	11	3	0
1	A	B					27	10	3	0	0
0	X					16	5	1	0	0	0
	0	1	2	3	4	5	6	7	8	9	10

Fig. 2. The matrix used for statistical tests. Probabilities are given in parts per thousand. Only p-values ≤ 0.032 are defined as statistically significant and indicated in the graph (the squares of the left upper and the right lower corners of the matrix). Statistically nonsignificant areas of the matrix are indicated by shading. The matrix is used for evaluating the probability that a given sequence by which the ants carry pupae of two types to their nest is due to chance. An example of the procedure is shown in which the carrier ants do not show any preference for pupae of either the type A or the type B. The trajectory, within the coordinate system, formed by the sequence in which A and B pupae are carried to the recipient nest does consequently not enter any of the two low-probability areas of the graph. Removal of a pupa of the type A shifts the row one step along the vertical axis, while removal of one pupa of the type B shifts the row one step along the horizontal axis. The encircled A-capital marks the point where a total of 10 pupae of either type had been carried away. This point is represented by number 6 on the vertical axis, corresponding to the recognition index for pupae of the type A (see the text for further explanations).

the matrix of Fig. 2). Secondly, we calculated a brood preference index based on the location reached in the matrix (Fig. 2) at the point when 10 pupae (of either kind) had been carried away. The vertical axis represents the pupae of the "right" kind, those that we expected the ants to recognize (note that we always give the indices in terms of the vertical axis). The highest score, 10, means that all carried pupae were of the "right" kind while the lowest, 0, means that the first 10 carried pupae were all of the "wrong" kind. The discrimination index was used to characterize groups of experiments when comparing different categories such as A and B.

For the experiments reported here, we used only live pupae of sexuals. This differs from previous tests, using the same method, in which only worker pupae were used (ROSENGREN, CHERIX 1981, SEEGER 1985). Our preliminary observations did not reveal any obvious tendency in the carrier ants to discriminate between male and female pupae or between older, pigmented pupae and young, still unpigmented ones.

RESULTS

Carrier ants of both the types A and B showed, in most cases, a statistically significant preference for pupae of their own electrophoretic type, while no case of preference for an alien electrophoretic type was recorded (Table 1, Fig. 3). This is clearly not explainable by nestmate recognition based on familiarity (sensu WALDMAN et al. 1988) or fellowship (JAISSON 1987, 1991). In 39 out of 49 cases in which recipient workers expressed a statistically significant discrimination against pupae of the other electrophoretic type (="heterotype" pupae) the ants were offered a choice between non-nestmate pupae of the same electrophoretic type (="homotype" pupae) and heterotype pupae (Fig. 3). Furthermore, there was no significant difference between the two combinations: nestmate (homotype) pupae vs. heterotype pupae and non-nestmate homotype pupae vs. heterotype pupae (Table 2), although the mean preference for "own" ones in the latter combination was lower (compare the mean indices in Table 2).

Table 1. The results of 85 choice tests using the "pupae-carrying" method. Data given separately for the A- and B-type nests. The three pupae combinations were: 1) pupae of the same electrophoretic type as the recipient, but from another nest (alien homotype), tested against pupae of the other electrophoretic type (heterotype), 2) nestmate pupae, i.e. pupae from the same sorce as the recipient (own homotype), tested against pupae of the other electrophoretic type (heterotype) and 3) nestmate pupae (own homotype) tested against pupae originating from other nests of the same electrophoretic type (alien homotype). Row and column sums are given (in italics).

Recipient nest	Combination of pupae	Prefers own	Prefers alien	Neutral	
A	alien A vs. B	26	0	5	*31*
	own A vs. B	5	0	2	*7*
	own A vs. alien A	2	0	12	*14*
B	alien B vs. A	13	0	5	*18*
	own B vs. A	5	0	0	*5*
	own B vs. alien B	2	0	8	*10*
		53	*0*	*32*	*85*

It may, on the other hand, be premature to conclude that our results refute all traces of nestmate recognition vis à vis pupae. In 4 out of 24 experiments (data from both types combined), the ants preferred to carry nestmate pupae to alien homotype pupae (Table 1, Fig. 3). It should be noted that the word "preference" here refers to a statistically significant bias and that no case of preference in the opposite direction was observed.

The Kruskal-Wallis one-way analysis of variance by ranks, based on the mean preference indices for different nests, revealed a slight but statistically significant effect of individual recipient nests (H corrected for ties = 18.229, p = 0.0326). Most important, however, this effect was not associated with the electrophoretical type of the nest; recipients of the type A or the type B did not differ significantly with respect to discrimination ability (comparing the recipients of the type A to the recipients of the type B by Mann-Whitney rank sum test; U = 374.5, Z corrected for ties = –1.392, p = 0.1639).

Table 2. The mean discrimination indices of binary combinations of pupae from different types of donor nests. The combinations are the same as explained in the legends of Fig. 3 and Table 1 (and have been denoted with the same code numbers 1–3). Shown are the arithmetic means, the standard deviations (SD), the numbers of tests (n) and the mean ranks used in the Mann-Witney rank sum test. This nonparametric tests give significant differences for the combinations 1 vs. 3 and 3 vs. 2 but not for 1 vs. 2.
The same data were treated also with a parametric one-way ANOVA after a combined arcsin and square root transformation of the original index scores (overall $F = 21.062$; $p = 0.0001$). The Scheffé F-test for pairwise comparisions gave nonsignificant differences for 1 vs. 2 (Scheffé $F = 1.799$), but significant differences, at the 95% level, for 1 vs. 3 (Scheffé $F = 15.237$) and 3 vs. 2 (Scheffé $F = 15.783$). The results of the nonparametric and the parametric tests thus coincided.

Combination of pupae	Mean index	SD	n	Mean rank	U	p
1. homotype vs. heterotype	7.9	1.36	49	45.8	156	0.0001
3. nestmate vs. homotype	5.7	1.30	24	19		
1. homotype vs. heterotype	7.9	1.36	49	29.4	216	0.146 (n.s.)
2. nestmate vs. heterotype	8.6	1.38	12	37.5		
3. nestmate vs. homotype	5.7	1.30	24	13.4	21.5	0.0001
2. nestmate vs. heterotype	8.6	1.36	12	28.7		

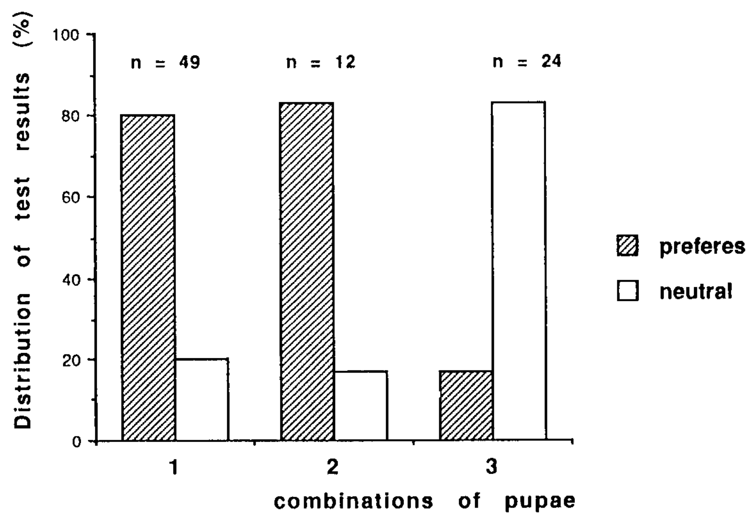


Fig. 3. The percentual distribution of test results in which the carried ants of recipient nests were offered a choice between different categories of pupae (data from recipients of the type A and B have been pooled). Shaded columns: tests in which the workers significantly prefered pupae of their own kind. White columns: test in which the workers did not show a preference for pupae of either kind. The third alternative: workers preferring alien pupae to pupae of their own type, did not occur (see also Table 1) and is consequently not included in the graph. The graph gives data for three combinations of donors: 1 – alien homotypes vs. heterotypes, 2 – own (nestmate) homotypes vs. heterotypes, 3 – own (nestmate) homotypes vs. alien homotypes; n – number of tests.

We may summarize this section by concluding that nestmate recognition in the behavioural context of brood retrieval, if occurring at all, is of minor importance compared to "homotype recognition" (Fig. 3, Tables 1, 2).

According to some previous studies the extent to which conspecific ants from other nests are accepted as nestmates is negatively correlated to the linear distance between donor and recipient nests (BENNETT 1986, ROSENGREN et al. 1986). We plotted the brood preference index against the distance between the recipient nest and the homotype donor, in the experiments where non-nestmate homotype pupae were tested against heterotype pupae. No effect of distance on the preference for homotype pupae was detected (Fig. 4).

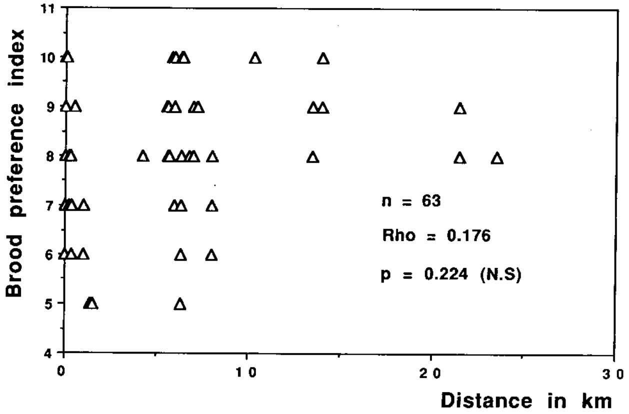


Fig. 4. Brood-discrimination-index as a function of the distance in km between the recipient nest and the homotype donor nest: *n* –number of tests, *Rho* = the Spearman rank correlation coefficient (SIEGEL 1956), *p* – the probability value based on the Spearman rank correlation test (*Z* corrected for ties = 1.217).

DISCUSSION

Supercoloniality and speciation

Colonies of polygynous *F. rufa* group species multiply their nests by budding. Groups of mature mound nests originated by this mechanism often remain connected by communication routes for years (ROSENGREN, PAMILO 1983). Different nests of a system may exchange enormous numbers of brood and young workers with a storage function, sometimes also mature queens (MARIKOVSKY 1962, our observations both from southern Finland and from the Jura site). Such networks of interconnected, highly polygynous nests, sometimes termed "supercolonies", may extend over vast areas, as exemplified by our study area in the Jura mountains (GRIS, CHERIX 1977, CHERIX 1980, 1987). It is understandable that intracolonial relatedness is extremely low (data summarized in ROSENGREN et al. 1993) and genetically correlated nestmate recognition poor or absent (BENNETT 1986, BREED, BENNETT 1987) within local populations of this type.

What is the relation, if any, between supercoloniality on the one hand and genetic differentiation and speciation on the other? It is conceivable that the fluent nesting and population structure often associated with supercoloniality (e.g. ROSENGREN et al. 1986) could inhibit genetic differentiation on a geographic microscale by "mixing" different parts of the local population. Such buffering against differentiation would be extended to a larger scale if, in addition, sexuals from different supercolonies form a more or less panmictic "breeding community".

On the other hand, however, it is conceivable that supercoloniality, which often means that the population of a species is divided to discrete patches of very high local density (HÖLLDOBLER, WILSON 1990), could be associated with constraints on the gene flow. Such constraints may occur if the probability that mated females are adopted in established nests (FORTELIUS et al. 1993) depends on their genotype, if matings occur on the nests or are confined to more or less restricted lek areas (CHERIX et al. 1989, 1991, 1993, WALTER et al. 1993) or if killing of mature queens by workers from other nests of the same supercolony (ROSENGREN, PAMILO 1983) is a selective process. Those processes would enhance genetic differentiation between different supercolonies of a species.

It is also possible that cumulative genetic differentiation takes place within a supercolony. For instance, we may imagine that the differentiation process is initiated by groups of nests at the periphery of the supercolony experiencing a higher gene flow from alien sources. Evolved differences in recognition cues could be further amplified by olfactory barriers against exchange of brood with other parts of the supercolony. It is thus conceivable that a combination of sexual and social mechanisms could lead to genetic differentiation on several different levels of the population hierarchy.

The finding of socially isolated, hostile nest groups characterized by a deviant hydrocarbon pattern in workers (CHERIX 1983) within the area of the original supercolony of the Jura mountains (GRIS, CHERIX 1977) appears to lend some support to the above idea of local speciation. However, the genetic data of PAMILO et al. (1992), indicating a close genetical similarity between the type A and the form of *F. lugubris* occurring on the British Isles, do not support any local origin of the genetic/taxonomic division between the types A and B. Those results would rather suggest an explanation based on the colonization history of the Jura mountains. It may therefore be more reasonable to ask how the required genetic isolation between the two taxa can be maintained today.

The nature of the discrimination mechanism

In order to recognize the olfactory characteristics of their brood, *F. rufa* group workers have to learn them during a sensitive period (JAISSON 1975, 1987, 1991). This early learning or imprinting process occurs in other studied ant species as well, although the data in some cases indicate preprogrammed learning, biasing the process toward learning the labels of conspecifics first of all (HARE, ALLOWAY 1987, CARLIN et al. 1987, CARLIN 1988) That a behavioural signal has to be learned does not of course imply that the label itself is a contextual marker acquired from the environment (e.g. food flavours or odours emanating from the nest material and absorbed in the epicuticle).

It could be argued that the ants' ability to discriminate the type A from the type B pupae represents environmentally acquired labels due to the location of the nests in different habitat types. This hypothesis does not appear plausible for the following reasons: 1) The nests of A and B occur partly intermingled within the distribution area (Fig. 1, see also Fig. 4 in PAMILO et al. 1992) and thus are not in vegetationally different areas; 2) The area, and altitude, in which the nests occur represents a geologically, climatologically and vegetationally fairly homogenous area.

It has furthermore been argued that extension of social acceptance to non-nestmates may be attributable to mixing of odour cues due to interchange of workers and brood between nests in a polycalic species (MARIKOVSKY 1962). This mechanism cannot explain our present findings, because each "homotype" included socially isolated patches separated by distances of up to 20 km (Figs 1, 4). It is more likely that the close correlation between our behavioural tests and the two electrophoretical types reflected genetically mediated differences in brood recognition cues.

There is evidence that both contextual markers acquired from the environment and genetically mediated cues may contribute to recognition in other ant species (BENNETT 1986, BREED, BENNETT 1987, CARLIN 1988, HÖLLDOBLER, WILSON 1990, JAISSON 1991). However, only the role of contextual markers has so far been pointed out in the case of wood ants (e.g. MABELIS 1979). Indirect evidence based on transfer of colourmarked workers between nests of *Formica* s.str. (ROSENGREN, PAMILO 1983, ROSENGREN et al. 1986, ROSENGREN, unpubl.) suggest, like the result of the present study, that genetically mediated labels may indeed be important for recognition and discrimination both within and between species of the *F. rufa* group.

The brood recognition observed in this study may be mediated by genetically determined hydrocarbon profiles in the epicuticle of the insects (BONAVITA-COUGOURDAN et al. 1987, BLUM 1987). Such hydrocarbon "clothings" (BLUM 1987) have recently been implicated also in the case of brood recognition in ants (BONAVITA-COUGOURDAN et al. 1988).

The ability to distinguish between nestmate brood and conspecific brood of non-nestmates has been demonstrated in *Cataglyphis cursor* FONSC. (LENOIR 1984, ISINGRINI et al. 1985) in *Camponotus vagus* (SCOP.) (BONAVITA-COUGOURDAN et al. 1988) and in *C. floridanus* (BUCKL.) (CARLIN, SCHWARTZ 1989). Experiments with *Rhytidoponera confusa* (FOR.) did not, on the other hand, provide any evidence for kin- or nestmate recognition and the ants even proved unable to distinguish conspecific brood from brood of other species within that genus (CROSLAND 1988).

The present result gave circumstantial evidence of a bias in favour of nestmate brood when compared to non-nestmate brood of the same electrophoretic type. Previous results with the same method did not show any such bias and, in some cases, the ants, even proved unable to distinguish between closely related, morphologically different taxa within the same subgenus (ROSENGREN, CHERIX 1981, SEEGER 1985, ROSENGREN, unpubl.). The difference between our present experiments and the previous ones was that we exclusively used pupae of sexuals while only worker pupae were used in the previous studies. The inclusive fitness theory predict that workers should be more choosy towards sexual brood than towards worker brood (ROSENGREN et al. 1993), but we do not yet have enough data to confirm (or refute) this proposition.

True kin recognition may be defined as the process by which individuals assess the genetic relatedness of conspecifics to themselves or others (WALDMAN et al. 1988). It is not clear whether true kin recognition, in this sense, exists in ants, or whether nestmate recognition is a valid example of it. The phenomenon reported here does not imply an ability to measure the degree to which any individual is genetically related to any other, and hence does not represent kin recognition as defined above. It can, however, be argued that true kin recognition, nestmate recognition, "supercolony-recognition" and species recognition are parts of the same behavioural continuum for distinguishing "self" from "non-self". The phenomenon reported here clearly belongs to the higher-level end of this continuum, that is, to distinction among taxa.

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STRESZCZENIE

Wyodrębnianie dwóch sympatrycznych siostrzanych gatunków z rodzaju *Formica* L. za pomocą testu behawioralnego, opartego o rozpoznawanie potomstwa

W poprzedniej pracy (PAMILO et al. 1992) wykazano, że w obrębie populacji *Formica lugubris* ZETT. w Jurze (Szwajcaria) występują obok siebie (Fig. 1) dwie formy dobrze wyodrębnione genetycznie (pod względem częstości alloenzymów). Celem tej pracy było sprawdzenie, czy mrówki każdej z tych elektroforetycznie wyróżnionych form dysponują swoistym dyskryminatorem, pozwalającym na wzajemne rozpoznawanie się. Wykonano to za pomocą testu behawioralnego, tzw. "metody noszenia poczwarek" (ROSENGREN, CHERIX 1981), polegającego na podawaniu mrówkom do wyboru równomiernie rozmieszczonych na arenie poczwarek konspecyficznych dwojakiego typu i rejestrowaniu kolejności wnoszenia ich do gniazda przez robotnice. Do testu wykorzystano poczwarki form seksualnych. W 20 spośród 24 powtórzeń testu robotnice-biorcy nie przejawiały istotnej preferencji wobec własnych poczwarek w porównaniu z poczwarkami tego samego typu elektroforetycznego, ale pochodzącymi z innego gniazda. Zarazem, w 39 z 49 powtórzeń, mrówki wykazały statystycznie istotną preferencję wobec obcych poczwarek ich własnego typu w porównaniu z poczwarkami obcego typu elektroforetycznego (Tab. 1, Fig. 3). Rezultaty zestawiono, obliczając średni wskaźnik preferencji dla każdej z kategorii testów (Tab. 2). Nie stwierdzono żadnej korelacji między wielkością wskaźnika preferencji a fizyczną odległością między gniazdami "dawcą" i "biorcą" (Fig. 4). Mrówki różnych typów nie różniły się istotnie między sobą możliwością rozróżniania poczwarek "własnych" i "obcych". Wyniki, potwierdzając przypuszczenie, że mrowiska dwóch typów elektroforetycznych reprezentują gatunki siostrzane (PAMILO et al. 1992), wskazują zarazem, że dyskryminatory potomstwa są w tym przypadku uwarunkowane genetycznie. Uzyskane rezultaty są omawiane zarówno pod kątem hipotetycznego procesu specjacji mrówek poliginicznych i polikalicznych, jak i domniemanej natury związków chemicznych zaangażowanych w zjawiska związane z rozpoznawaniem się osobników. Jak wykazano, bariery zapobiegające wymianie potomstwa między mrowiskami w obrębie superkolonii mogą ułatwiać genetyczne różnicowanie się w skali mikrogeograficznej. Zdolność rozróżniania potomstwa może mieć natomiast swój udział w procesie specjacji.